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# Topoisomerase II Poisons: Converting Essential Enzymes into Molecular Scissors

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## Abstract

The extensive length, compaction, and interwound nature of DNA, together with its controlled and restricted movement in eukaryotic cells, create a number of topological issues that profoundly affect all of the functions of the genetic material. Topoisomerases are essential enzymes that modulate the topological structure of the double helix, including the regulation of DNA under- and overwinding and the removal of tangles and knots from the genome. Type II topoisomerases alter DNA topology by generating a transient double-stranded break in one DNA segment and allowing another segment to pass through the DNA gate. These enzymes are involved in a number of critical nuclear processes in eukaryotic cells, such as DNA replication, transcription and recombination, and are required for proper chromosome structure and segregation. However, because type II topoisomerases generate double-stranded breaks in the genetic material, they also are intrinsically dangerous enzymes that have the capacity to fragment the genome. As a result of this dualistic nature, type II topoisomerases are the targets for a number of widely prescribed anticancer drugs. This article will describe the structure and catalytic mechanism of eukaryotic type II topoisomerases and will go on to discuss the actions of topoisomerase II poisons, which are compounds that stabilize DNA breaks generated by the type II enzyme and convert these essential enzymes into “molecular scissors.” Topoisomerase II poisons represent a broad range of structural classes and include anticancer drugs, dietary components, and environmental chemicals.

## Graphical Abstract

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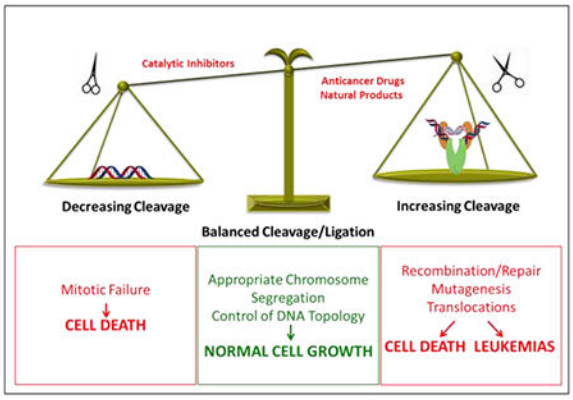


## Introduction.

The genetic information required to encode the human genome is stored in approximately two meters of DNA, which is severely compacted within the nucleus of the cell (5-10 μm in diameter).[1] The DNA contains two complementary polynucleotide strands that are plectonemically coiled, wrapped around a histone core to form nucleosomes, and further condensed into higher order chromatin and chromosome structures.[1] Interactions with scaffolding proteins, together with the extreme length of the genome, hinder the free rotation of the DNA strands. These properties, together with the interwound nature and restricted rotation of DNA, create a number of topological issues that profoundly affect all of the functions of the genetic material.[2–6] Topological relationships within DNA, including under- and overwinding (*i.e.*, negative and positive supercoiling, respectively), tangling, and knotting can be altered only by breaking one or both strands of the double helix.[4, 7]

## DNA Topology.

Human DNA is globally underwound by ~6%.[2, 7, 8] This has important ramifications because duplex DNA acts as the storage form of the genetic information. In order to replicate or express this information, the two strands of DNA must be separated to gain access to the genetic material. DNA underwinding decreases the energy needed to break the hydrogen bonds between complementary bases, thereby facilitating strand separation.[2, 8, 9]

In contrast to basal levels of negative supercoiling, hyper-negative supercoiling that occurs behind transcription complexes promotes the formation of R-loops, which is a source of genomic instability.[10–12] Furthermore, positive supercoiling creates significant problems for DNA systems that require helicases, such as replication and transcription.[2, 4, 7, 8] Because helicases separate the two strands of DNA without unwinding them, they do not remove any of the turns of the double helix.[2, 4, 8, 9] Consequently, these DNA turns are compressed ahead of the helicase, overwinding the double helix and generating severe torsional stress in the genetic material. Unless this torsional stress is alleviated, the replication and transcription machinery can no longer track along the DNA and these essential nuclear processes will stall rapidly.

DNA replication also generates intermolecular tangles (*e.g.*, catenanes) between daughter chromosomes.[2, 8] Unless these tangled DNA molecules are decatenated, daughter chromosomes cannot be segregated properly during mitosis or meiosis. Similarly, intramolecular knots formed within the same DNA molecule are generated during recombination.[2, 8] Because DNA knots make it impossible to completely separate the two strands of the double helix, they block essential nucleic acid processes.[4] Consequently, DNA tangles and knots can be lethal to cells if they are not resolved.[2, 4, 7, 8]

## Topoisomerases.

Topoisomerases regulate the topological structure of the genetic material during cellular processes. These enzymes are essential for the survival of all organisms and alter DNA topology by generating transient breaks in the double helix.[7, 8, 13–19]

Topoisomerases can be separated into two major classes, type I and type II, based on the number of DNA strands that they cleave and ligate during their catalytic cycles.[7, 8, 13–18, 20] Type I enzymes (denoted by odd numbers) transiently cleave a single strand of the DNA duplex, while type II enzymes (denoted by even numbers) cleave both strands. Because type I topoisomerases cleave only a single strand, they can modulate DNA supercoiling, but cannot remove knots or tangles from intact duplex DNA.[4, 7, 8] In contrast, because type II topoisomerases cleave both strands of the double helix, they can regulate DNA under- and overwinding and can also remove tangles and knots from the genome.[4, 7, 8, 13, 21]

## Type II Topoisomerases.

Eukaryotic type II topoisomerases function as homodimers and require ATP and divalent metal ions for overall catalytic activity.[20–23] Briefly, they interconvert different topological structures by generating a transient double-stranded break in the DNA backbone, transporting a separate double helix through the nucleic acid gate, and resealing the break. Both of the protomer subunits of topoisomerase II contain an active site tyrosine residue, which cuts the DNA by a nucleophilic attack on the sugar-phosphate backbone.[20, 21] In order to maintain the integrity of the genome and conserve the bond energy of the backbone while the DNA is cleaved, type II enzymes covalently attach to the newly generated 5'-terminal phosphates (4-base pairs apart on opposite strands).[8, 13–17, 20, 24] This covalent enzyme-cleaved DNA complex, the *cleavage complex*, is a hallmark of all type II topoisomerases and contains a phosphotyrosine-linked protein and a free deoxyribose hydroxyl on the 5'- and 3'-termini, respectively.[8, 13–17, 20]

All living organisms encode at least one type II topoisomerase.[7, 8, 17, 20] Lower eukaryotes encode a single type II enzyme, while vertebrates express two isoforms of topoisomerase II, α and β.[16, 21, 25] Human topoisomerase IIα and IIβ are encoded by two separate genes (17q21–22 and 3p24, respectively) and differ in molecular mass (170 and 180 kDa, respectively). Both isoforms share extensive sequence identity (70%) and display similar enzymatic properties, but differ significantly in their expression, cellular regulation, and functions.[14, 16, 17, 21, 25–27]

Expression of topoisomerase IIα is linked to cellular growth and is essential for the survival of proliferating cells.[4, 13, 14, 16, 21, 25] Although the α isoform is virtually non-existent in quiescent and differentiated tissues, rapidly proliferating cells contain ~500,000 molecules.[18, 25, 27, 28] Topoisomerase IIα is associated with replication forks and remains tightly bound to chromosomes during mitosis. The enzyme plays roles in DNA transcription and recombination,[18, 29] is important for fork convergence and termination of replication,[30] and is required for proper chromosome organization and segregation.[8, 18, 29, 30]

In contrast to topoisomerase IIα, the β isoform is not required at the cellular level.[25] However, it is essential for neuronal development.[7, 16, 20, 25, 31] High levels of the β isoform are found in most cell types, independent of proliferation status.[7, 18, 25, 32] Topoisomerase IIβ dissociates from chromosomes during mitosis, but appears to play an important role in the transcription of hormonally and developmentally regulated genes.[25, 33–35] Despite the differences between the α and β isoforms of topoisomerase II, these enzymes are mechanistically and structurally similar and will be collectively referred to in this article as topoisomerase II, unless otherwise noted.

## Structural Domain Organization of Eukaryotic Topoisomerase II.

Each topoisomerase II protomer contains three main regions: the N-terminal region, the catalytic core, and the C-terminal region (Figure 1).[20, 21] The N-terminal region contains the N-gate (through which the DNA enters the enzyme), the GHKL (Gyrase, Hsp90, Histidine Kinase, MutL) domain (also known as the ATPase domain) that binds and hydrolyzes ATP, and the transducer domain that relays hydrolysis signals to the catalytic core of the enzyme.[20, 21] The catalytic core contains the TOPRIM (topoisomerase/primase) domain that coordinates the active site divalent metal ions, the WHD (winged-helix domain) that includes the active site tyrosine, and the tower domain that makes polar and electrostatic contact with the DNA.[20, 21] The C-terminal region (CTR) contains nuclear localization signals and post-transcriptional modification sites.[20, 21, 37] The CTR is largely disordered in the protein when it is not bound to DNA and is poorly conserved among the type II enzymes.[20, 21] It is not necessary for enzyme function, but it contains protein elements that allow topoisomerase IIα to discern the geometry of DNA supercoils and remove positive supercoils ~10 times faster than it does negative supercoils.[8, 38, 39]

## Catalytic Mechanism of Topoisomerase II.

Topoisomerase II alters DNA topology using the double-stranded DNA passage reaction, which is shown as a series of discrete steps in Figure 2. 1) The enzyme starts its catalytic cycle by binding two DNA segments at a crossover.[40] The first segment bound by the enzyme is the double helix that will be cleaved by the enzyme and is referred to as the “Gate-” or “G-segment.”[41] The second segment is the double helix that will be transported through the transiently cleaved G-segment and is referred to as the “Transport-”or “T-segment.”[40, 42, 43] 2) In the presence of divalent metal ions (coordinated within the TOPRIM domain), the type II enzyme samples the G-segment for bendability.[22, 44–46] DNA sequences that can be bent by the enzyme[45, 47, 48] are distorted to an angle of ~150° and can be used as a site for enzyme action.[41] 3) Cleavage of the bent G-segment is catalyzed by the

nucleophilic attack of the active site tyrosine residues to form the cleavage complex.[41, 44] The two tyrosine residues act in a coordinated manner. Once the first strand has been cleaved, the second is cleaved >10-fold faster.[49] 4) Upon the binding of two ATP molecules, the enzyme closes the N-gate, forcing the T-segment through the open DNA-gate.[13, 20, 21] The DNA strand passage event appears to take place more rapidly if one of the ATP molecules is hydrolyzed.[50] 5) The G-segment is then ligated, and the T-segment is released.[13, 20, 21] 6) Following hydrolysis of the second ATP molecule, the G-segment can be released and 7) the enzyme conformation is reset, allowing for the capture of another DNA crossover.[13, 20, 21]

## Converting Type II Topoisomerases into Molecular Scissors.

Because type II topoisomerases generate double-stranded DNA breaks as part of their reaction mechanism, they are intrinsically dangerous enzymes.[13, 15, 18] Although essential to cell viability, type II topoisomerases also have the capacity to fragment the genome (Figure 3). As a result, levels of cleavage complexes are maintained in a critical balance that greatly favors ligation. Under normal circumstances, these complexes are short-lived and are readily reversible.[13, 15, 18, 20] However, compounds that stabilize cleavage complexes have serious cellular consequences.[13–18, 46]

When levels of cleavage complexes, reflecting overall levels of enzymatic activity, drop below threshold concentrations, daughter chromosomes remain entangled following replication. As a result, sister chromatids cannot properly segregate during mitosis and cells die due to catastrophic mitotic failure.[8, 20] When levels of cleavage complexes rise to critical levels, cells also die, but for a different reason. In this instance, cell death is due to the conversion of transient DNA cleavage intermediates to strand breaks that can no longer be ligated by topoisomerase II and have to be rejoined by recombination/repair pathways.[13, 15, 18, 20, 51, 52] These breaks are believed to be generated when replication forks, transcription complexes, or other DNA tracking enzymes approach or attempt to traverse the covalently bound protein ‘roadblock’ in the genetic material.[13, 52–54] The mechanism that renders topoisomerase II incapable of resealing the DNA breaks has not yet been defined. It may be direct protein interactions with the type II enzyme or extreme DNA overwinding generated by oncoming DNA tracking systems. The conversion of trapped cleavage complexes to untethered DNA breaks is complex and involves the actions of DNA repair/processing enzymes.[55–59] Ultimately, the resulting damage and induction of recombination/repair pathways can generate a variety of mutations and chromosomal aberrations. When topoisomerase II-generated breaks are present in sufficient numbers, they can overwhelm the cell and initiate cell death pathways.[13, 52–54]

Because of this potentially lethal property of topoisomerase II, the enzyme has become a prominent target for the development of anticancer drugs.[13, 15, 54] However, in some cases, the use of these drugs initiates chromosomal translocations that trigger specific types of leukemias in ~2-3% of patients.[18, 53, 60–64]

## Topoisomerase II Catalytic Inhibitors *vs*. Poisons.

Compounds that alter topoisomerase II activity can be divided into two categories: *catalytic inhibitors* and *poisons* (Figure 4). Topoisomerase II catalytic inhibitors are compounds that impair the overall catalytic activity without increasing the concentration of cleavage complexes.[49, 54, 65, 66] Inhibitors have been described that act at a variety of different steps of the topoisomerase II catalytic cycle, including DNA binding, ATP binding, ATP hydrolysis, and DNA cleavage.[65–69] Depending on which step of the catalytic cycle is targeted, inhibitors may have very different cellular effects. For example, an inhibitor that blocks topoisomerase II-DNA binding will rob the cell of all of the essential functions of the enzyme, including structural functions. Alternatively, an inhibitor that blocks ATP binding will rob the cell of the catalytic functions of topoisomerase II, while an inhibitor that blocks ATP hydrolysis will freeze the N-terminal gate in a closed conformation, trapping topoisomerase II on the DNA and blocking critical nucleic acid processes.[65–69]

Topoisomerase II poisons are compounds that increase levels of topoisomerase II-DNA cleavage complexes. The term “poison” was originally applied to these compounds because they convert the enzyme into a cellular toxin that initiates the mutagenic and lethal consequences described above.[13, 15, 54, 68] Compounds that act in this manner are referred to as topoisomerase II poisons irrespective of whether or not they inhibit enzyme catalytic activity. This is because the increased levels of cellular DNA breaks induced by these compounds represent a gain-of-function, dominant phenotype.[13, 15, 54, 68] All clinically relevant topoisomerase II-targeted drugs identified to date act as poisons and interfere with the ability of the enzyme to religate cleaved DNA molecules.[13, 15, 54, 70, 71]

Topoisomerase II inhibitors and poisons are often confused in the literature. However, considering the dramatically different effects that catalytic inhibitors and poisons exert on the type II enzyme and on the cell, it is important to understand the differences between the two classes of compounds and intellectually keep them separate.

It is also important to recognize that many topoisomerase II poisons can cure or prevent cancers. Therefore, despite the negative connotations associated with the term “poison,” these compounds have a considerable number of positive attributes. Put another way, just because something is called a poison does not mean that it is necessarily bad for you.

## Mechanisms of Topoisomerase II Poisons.

Topoisomerase II poisons act by two distinct mechanisms: interfacial *vs*. covalent (Figure 4). *Interfacial poisons* interact in a non-covalent manner at the topoisomerase II-DNA interface within the vicinity of the active site tyrosyl residues.[15, 17, 71] These compounds are bi-functional and must interact with both the protein and the DNA in order to increase levels of DNA scission. Upon DNA cleavage, interfacial poisons insert between the ends of the double helix at the cut scissile bonds on the G-segment.[13, 15, 17, 54, 71] They distort the active site within the cleavage complex and appear to detach one of the catalytic magnesium ions from the phosphotyrosyl moiety.[72] More importantly, interfacial poisons act as physical barriers to ligation, functioning as “molecular doorstops.”[13, 54, 73] In order to stabilize

double-stranded breaks, two molecules (one at each scissile bond) must be present in the active site of the type II enzyme. Etoposide (Figures 4 and 5) is a classic example of an interfacial topoisomerase II poison.

Kinetic, drug-enzyme binding, and saturation transfer difference [$^1$H]-nuclear magnetic resonance spectroscopy studies all indicate that interfacial poisons contact topoisomerase II in addition to the DNA.[72–77] Despite the specific contacts that interfacial poisons make with both the protein and the cleaved DNA, they are surprisingly diverse in structure (Figure 5). Generally, but not always, they contain a multi-ring core with aromatic elements as well as a pendant ring.[78, 79] However, based strictly on the structure of a compound, it is difficult to predict whether it will act as a topoisomerase II poison.

In contrast to interfacial topoisomerase II poisons, *covalent poisons* contain protein-reactive groups and covalently adduct the enzyme at amino acids outside of the active site.[13, 80, 81] Most covalent poisons incorporate sulfhydryl-reactive groups such as quinones or maleimides and modify the enzyme through an acylation reaction.[80–86] Benzoquinone (Figures 4 and 6) is a classic example of a covalent topoisomerase II poison.

Covalent topoisomerase II poisons can be distinguished from interfacial poisons by a number of unique properties (Figure 4). First, because covalent poisons contain reactive groups, their ability to poison topoisomerase II can be abolished by the presence of reducing or thiol-containing agents.[86–88] Second, covalent poisons require the N-terminal region of the enzyme in order to exert their effects.[43, 80, 84, 85] Studies suggest that these compounds increase topoisomerase II-mediated DNA cleavage, at least in part, by closing the N-terminal gate.[84, 89] Consequently, in contrast to interfacial poisons, covalent poisons are unable to enhance DNA cleavage mediated by the catalytic core of topoisomerase II.[43, 88] Third, although covalent poisons enhance DNA cleavage when added to the topoisomerase II-DNA complex, they display the distinguishing feature of inhibiting enzyme activity when incubated with the enzyme prior to the addition of DNA.[80, 83, 84, 87, 88, 90–93] This feature may reflect the fact that closing the N-terminal protein gate prevents DNA binding.[80, 89] However, there is also evidence that covalent poisons adduct an essential reactive residue that is exposed (or unprotected) in the absence of DNA.[80, 85] Finally, while interfacial poisons always act by inhibiting DNA ligation, results with covalent poisons are more equivocal. Ultimately, it is still an open question as to the precise mechanism by which covalent poisons increase the levels of topoisomerase II-DNA cleavage complexes.

## Topoisomerase II Poisons as Anticancer Drugs.

Topoisomerase II poisons represent a group of widely prescribed anticancer drugs.[13, 15, 17, 18, 54] Currently, six topoisomerase II-targeted agents are approved for use in the United States.[13, 15, 17, 18, 54] These drugs encompass a group of naturally derived and synthetic compounds and are used to treat a variety of human malignancies (Figure 5). Notably, etoposide, doxorubicin, and their derivatives are frontline therapies for a number of systemic cancers and solid tumors, including leukemias, lymphomas, sarcomas, breast cancers, lung cancers, neuroblastomas, and germ-cell malignancies.[13, 15, 17, 18, 54] In addition, mitoxantrone is used to treat breast cancer, acute myeloid leukemia (AML), non-

Hodgkin lymphoma, and multiple sclerosis. It is notable that all topoisomerase II-targeted anticancer drugs act as interfacial poisons.

Etoposide was one of the first topoisomerase II-targeted agents to be used clinically (Figure 5).[13, 15, 17, 18, 54, 94, 95] The drug is a semisynthetic derivative of podophyllotoxin, which comes from *Podophyllum peltatum* (also known as the mayapple or American mandrake plant) and has been used as a folk remedy for over a thousand years. [94, 96]

Etoposide is by far the best-characterized topoisomerase II poison.[13, 15, 17–19, 54, 94, 95] Extensive research on this anticancer agent has provided a knowledge base that has paved the way for later drug studies. Etoposide was the first topoisomerase II poison that was demonstrated to inhibit the DNA ligation activity of the type II enzyme.[70] Furthermore, the drug was shown to enter the binary enzyme-DNA complex primarily through interactions with the protein.[74] Recent data has revealed structure-function relationships for the interaction of etoposide with topoisomerase II that are predictive with regard to new drug design.[72, 73, 97, 98]

Although topoisomerase II-targeted drugs represent important anticancer agents, several also appear to trigger chromosomal translocations that lead to the formation of specific leukemias. About 2-3% of patients treated with etoposide or doxorubicin eventually develop acute myeloid leukemia (AML) characterized by translocations with breakpoints in the mixed lineage leukemia (*MLL*) gene at chromosomal band 11q23.[18, 53, 61, 62, 64, 95] Furthermore, the increased use of mitoxantrone to treat breast cancer and multiple sclerosis is associated with the development of acute promyelocytic leukemia (APL) characterized by chromosome 15:17 translocations involving the promyelocytic leukemia gene (*PML*) and the retinoic acid receptor α (*RARA*) genes.[18, 60–62, 99] In all cases, chromosomal breakpoints are located in close proximity to sites of topoisomerase II-mediated DNA cleavage. In the case of APL patients, ~60% of the breakpoints that have been sequenced reside within an 8-bp region that centers on a mitoxantrone-induced topoisomerase II-DNA cleavage site.[18, 60–62, 99]

Etoposide quinone (Figure 5), a metabolite generated by phase I metabolism, has been implicated in the generation of etoposide-induced AMLs.[18, 100–103] Etoposide is converted to a catechol by CYP3A4 in the liver, which is carried in the blood throughout the body. The catechol is converted to the quinone in the oxidizing environment of the hematopoietic system.[18, 101–103] In contrast to the parent drug, which acts as an interfacial poison, etoposide quinone enhances topoisomerase II-mediated DNA cleavage by acting primarily as a covalent poison.[92, 104, 105]

Although topoisomerase IIα and topoisomerase IIβ are both targets for anticancer drugs, mounting circumstantial evidence indicates that topoisomerase IIβ is the isoform primarily responsible for initiating drug-induced leukemias.[18, 54, 63, 64, 106] The initial evidence supporting this hypothesis came from a mouse skin carcinogenesis model in which the incidence of secondary malignancy was greatly diminished in skin-specific *top2*β–knockout mice.[107] Moreover, etoposide-induced DNA sequence rearrangements in cellular models required topoisomerase IIβ.[107]

Later evidence implicating topoisomerase IIβ in the generation of leukemias came from translocation models. Translocations require the juxtaposition of chromosomal partners and genes are expressed in transcription factories that bring multiple chromosomes into close proximity.[108, 109] Studies in cultured human cell lines indicate that *MLL* and its two most common translocation partners are found in the same transcription factory in multiple human cell lines[63, 64, 110, 111] and the majority of *MLL* breaks generated by etoposide treatment in these factories are dependent on the β isoform.[63] In addition, the genotoxic effects of etoposide in these cells appear to be mediated primarily by topoisomerase IIβ.[63]

Finally, because expression of topoisomerase IIα, but not topoisomerase IIβ, is proliferation dependent, differentiated tissues express the β isoform almost exclusively.[18, 25] Thus, some of the off-target toxicities of topoisomerase II-targeted drugs, including cardiomyopathy, are attributed (at least in part) to the β isoform.[18, 63, 106, 112] This has led to interest in the potential development of a drug that displays specificity for topoisomerase IIα, such as NK314 and ARN-21934.[18, 113, 114]

## Dietary Topoisomerase II Poisons.

Topoisomerase II poisons are regularly consumed as part of the human diet. These include bioflavonoids (flavones, isoflavones, and flavonols), catechins, catechols, isothiocyanates, and quinones (Figure 6).[81, 86, 90, 91, 115–118]

Bioflavonoids are a diverse group of polyphenolic compounds that are constituents of many fruits, vegetables, legumes, and plant leaves.[81, 119–122] Studies suggest that these compounds can help protect against cancer, cardiovascular disease, osteoporosis, age-related diseases, and inflammation.[81, 119–122] Although bioflavonoids exert a range of effects on human cells, a number of them are potent topoisomerase II poisons *in vitro* and *in cellulo*.[81, 100, 115, 119–124] Genistein, which is one of the most abundant isoflavones in soy, is perhaps the best-characterized bioflavonoid-based topoisomerase II poison (Figure 6).[81, 100, 115, 124] It acts as an interfacial poison, and its activity is dependent on the presence of a 4’-hydroxyl moiety on the B ring.[100, 115, 116] Genistein is highly active against both topoisomerase IIα and topoisomerase IIβ, and displays a potency and efficacy against the enzymes similar to that of etoposide.[100, 115, 124] In humans, genistein reaches micromolar concentrations in plasma following ingestion.[125]

Catechins are another important class of bioflavonoids with activity towards topoisomerase II.[100, 117] Green tea, which is one of the most commonly consumed beverages in the world, is a rich source of (−)-epigallocatechin gallate (EGCG) and related catechins (Figure 6). Diets rich in these compounds are associated with reduced risk of breast, prostate, colorectal, and lung cancer.[116, 122, 126] In contrast to genistein, EGCG is a covalent topoisomerase II poison.[116, 117] The mechanistic differences between bioflavonoids and catechins appear to be related to structural elements in the B and C rings. Although the C-4’ hydroxyl of the B ring is critical for bioflavonoids to act as interfacial poisons, the inclusion of two additional B ring hydroxyl groups increases redox activity and is required for catechins to act as covalent poisons. Because EGCG contains a non-aromatic C ring, it is unable to act as an interfacial topoisomerase II poison and functions exclusively as a

covalent poison.[116, 117] It is notable that the concentration of EGCG in human plasma and saliva is estimated to be as high as 4 and 48 μM, respectively, following consumption of ~3 cups of green tea.[127]

Olive plants are a rich source of catechols that are based on hydroxytyrosol (including oleuropein and verbascoside) (Figure 6).[128, 129] These compounds are powerful antioxidants that are converted to reactive quinones under oxidizing conditions.[81] Hydroxytyrosol, oleuropein, and verbascoside are found in all parts of the olive plant, including the leaves, bark, and fruit, as well as the oil pressed from the fruit. These metabolites are prevalent in the Mediterranean diet, which has great potential for cancer prevention.[130–132] Similar to other compounds with the potential to form quinones, hydroxytyrosol, oleuropein, and verbascoside act as covalent topoisomerase II poisons.[118] These compounds also enhance enzyme-mediated DNA cleavage in complex formulations intended for human dietary consumption, such as olive leaf extracts and olive oil.[118] Olive oil and hydroxytyrosol are currently in clinical trials as preventative measures for breast cancer (Clinicaltrials.gov Identifier NCT04174391 and NCT02068092).

Isothiocyanates, such as sulforaphane, are found in cruciferous vegetables, such as broccoli, cabbage, cauliflower, and kale (Figure 6) and are believed to have chemopreventative properties.[133] These compounds are reactive and also act as covalent topoisomerase II poisons.[86]

Curcumin is the principal flavor and color component of the spice turmeric (Figure 6).[134] In aqueous solution at physiological pH, it undergoes a spontaneous and complex autoxidation reaction that generates a series of quinone methide intermediates.[135] Although the parent compound and the stable bicyclopentadione end product display no activity toward topoisomerase II, the quinone oxidation intermediates are covalent poisons.[90, 136] Even in the complex formulation of turmeric, curcumin intermediates function as topoisomerase II poisons.[90] Beyond its culinary uses, curcumin is widely used in traditional Chinese herbal and Ayurvedic medicine to treat inflammation, bacterial infections, and cancers.[137–139] More than 100 clinical research trials have been initiated to investigate curcumin and its metabolites as an anticancer agent [Clinincaltrials.gov Identifiers: NCT04731844 (prostate cancer), NCT02724202 (colon cancer), NCT04208334 (head and neck cancer)].

Thymoquinone is the major bioactive compound in *Nigella sativa*, also known as black seed (Figure 6).[140] This Mediterranean plant has a rich history of medicinal use in Middle Eastern, Northern African, and Indian cultures that dates back more than 3,000 years.[140] Thymoquinone is a covalent topoisomerase II poison.[91] Black seed extract and oil, which are the medicinal form of the plant, also enhance topoisomerase II-mediated DNA cleavage.[91] Historically, black seed has been used to treat a variety of illnesses associated with inflammation, including asthma, bronchitis, fever, arthritis, and rheumatism. Recently, it has been shown to display anticancer activity in cellular and animal models.[140, 141] Following ingestion, its concentration in human plasma is in the micromolar range.[142]

6,6'-Dihydroxythiobinupharidine is the active ingredient in the *Nuphar leutea*, the yellow water lily. *N. leutea* has been used in traditional medicine by a variety of indigenous

populations.[143, 144] 6,6'-Dihydroxythiobinupharidine is a covalent topoisomerase II poison and appears to display selectivity for the α over the β isoform.[88]

Besides the treatment-related leukemias associated with topoisomerase II-targeted drugs, the only other malignancies that display 11q23 translocations are infant AMLs or acute lymphoblastic leukemias (ALLs).[18, 145] Approximately 80% of infants diagnosed with these leukemias display translocations in the *MLL* gene. The chromosomal translocations associated with these cancers have been observed *in utero*, indicating that infant leukemias are initiated during gestation.[18, 145] Epidemiological studies indicate that the risk of developing these leukemias increases more than 3-fold when pregnant women consume foods/drinks that are rich in dietary topoisomerase II poisons.[18, 53, 123, 146] Notably, treatment of cultured human cells with dietary bioflavonoids induces cleavage within the *MLL* gene.[123] Thus, while the consumption of topoisomerase II poisons in the human diet appears to be chemopreventative, it has the capacity to trigger leukemias in developing embryos.

## Environmental Topoisomerase II Poisons.

A variety of environmental quinone-based compounds that are damaging to human health are also potent topoisomerase II poisons (Figure 6).[83, 84, 87, 89, 147–150] All of these compounds act as covalent poisons. For example, 1,4-benzoquinone, a major metabolite of benzene, is associated with the development of leukemia.[150] Quinone metabolites of polychlorinated biphenyls (PCBs), which were used as industrial diluents, lubricants, and cooling fluids until the 1970s, are highly carcinogenic.[149] N-acetyl-*p*-benzoquinone imine (NAPQI), which is the toxic metabolite of acetaminophen, is known to be a potent liver toxin.[147] Finally, 1,2-naphthoquinone, a secondary metabolite of naphthalene, is an environmental pollutant found in diesel exhaust.[93]

## Summary.

In order to carry out their essential nuclear functions, type II topoisomerases generate transient double-stranded breaks in the genetic material. Consequently, although they are required for cell survival, type II topoisomerases are intrinsically dangerous enzymes. Topoisomerase II poisons exploit this hazardous property and convert the type II enzyme into "molecular scissors" that fragment the genome. Topoisomerase II poisons are structurally and mechanistically diverse, and the only apparent feature that some of them have in common is the ability to increase levels of topoisomerase II-mediated DNA cleavage. Although several topoisomerase II poisons can cure or prevent cancer in humans, these same compounds have been linked to the generation of specific leukemias. Although the catalytic mechanism of topoisomerase II has been well-studied, we still have much to learn about how topoisomerase II poisons alter enzyme activity and generate DNA damage. Hopefully, further research in the field will lead to the development of more effective anticancer drugs that display fewer harmful consequences.

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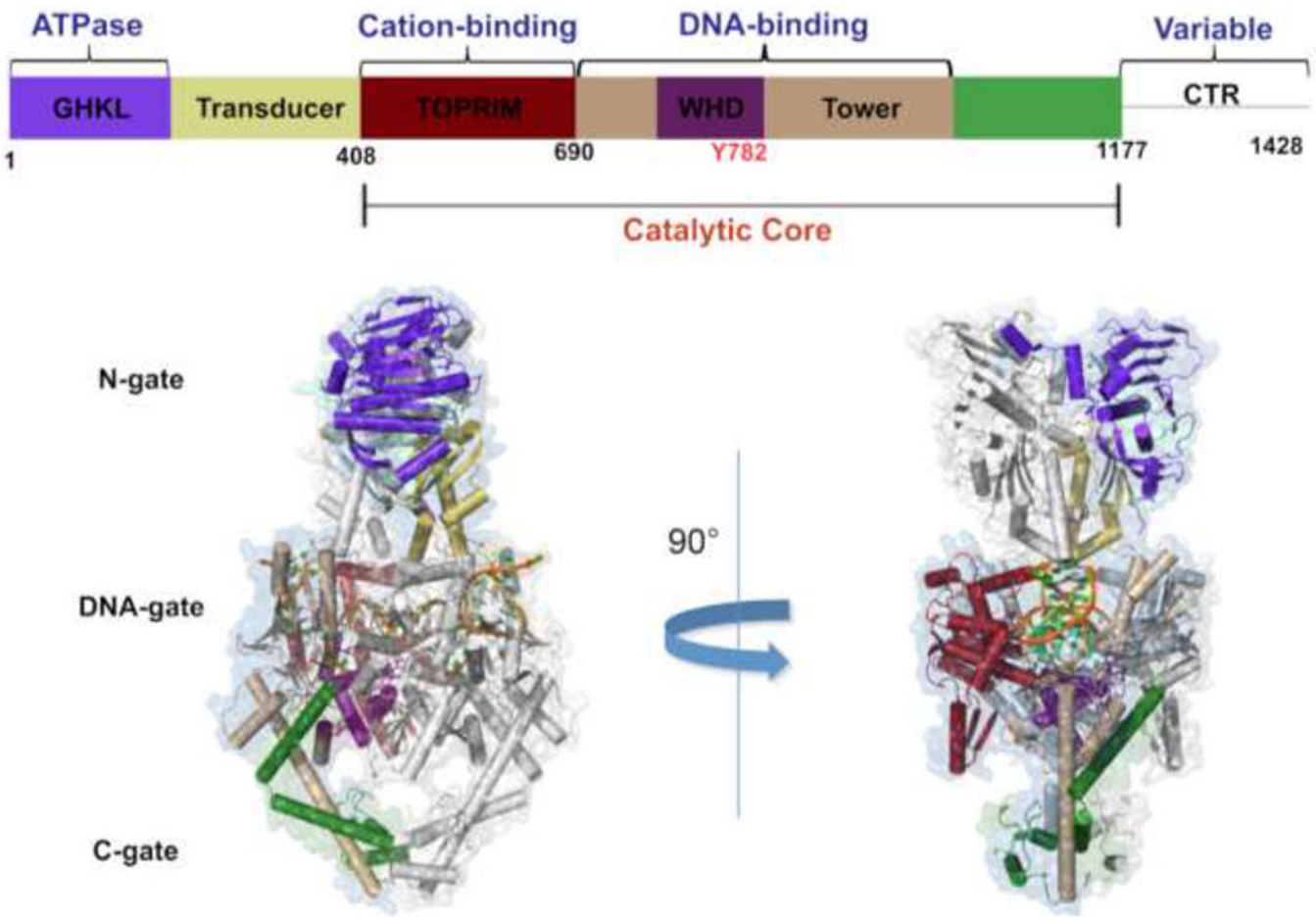


**Figure 1.**

Domain organization and structure of eukaryotic topoisomerase II. A schematic depicting the functional regions of a topoisomerase II protomer is colored and labeled above. The structure of a functional eukaryotic topoisomerase II from yeast is shown below in a covalent complex with double-stranded DNA. The PDB model 4GFH is shown and the functional regions are highlighted according to the schematic above for visualization.[36] One of the enzyme protomers is shaded gray to distinguish between the two protomers. The variable and unstructured C-terminal region (CTR) is not depicted in the structure.

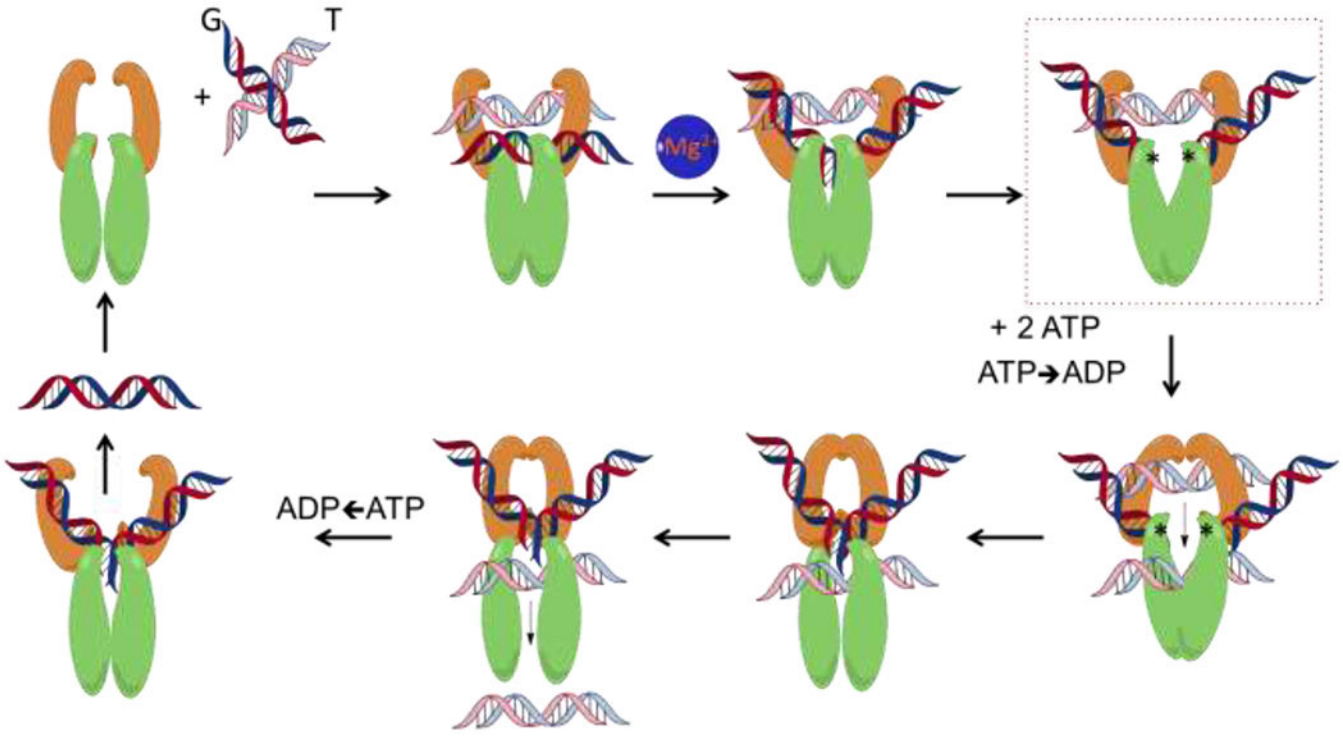


**Figure 2.**

The catalytic cycle of topoisomerase II. Type II topoisomerases regulate DNA topology by a multi-step process illustrated in the schematic. 1) The homodimer binds to DNA crossovers, positioning the first double helix as the DNA Gate-segment (G-segment) and the second as the Transport-segment (T-segment). 2) Upon the coordination of divalent metal ions in the TOPRIM region of the enzyme, the G-segment is sampled for bendability and bent. 3) The bent G-segment is then cleaved by nucleophilic attacks on the DNA backbone by the catalytic tyrosine residues, covalently linking the enzyme to the cleaved DNA (depicted by the asterisks) and allowing for the DNA-gate to open. This intermediate step represents the "cleavage complex" (highlighted by the dotted box). 4) Following the binding of two molecules of ATP in the ATPase domain, the N-gate closes, and hydrolysis of one ATP transduces signals through the enzyme allowing for rapid passage of the T-segment through the DNA-gate. 5) The opened DNA-gate is then resealed, 6) and the T-segment is released. 7) After hydrolysis of the second ATP molecule, the enzyme releases the G-segment, and the enzyme is reset to capture another crossover.

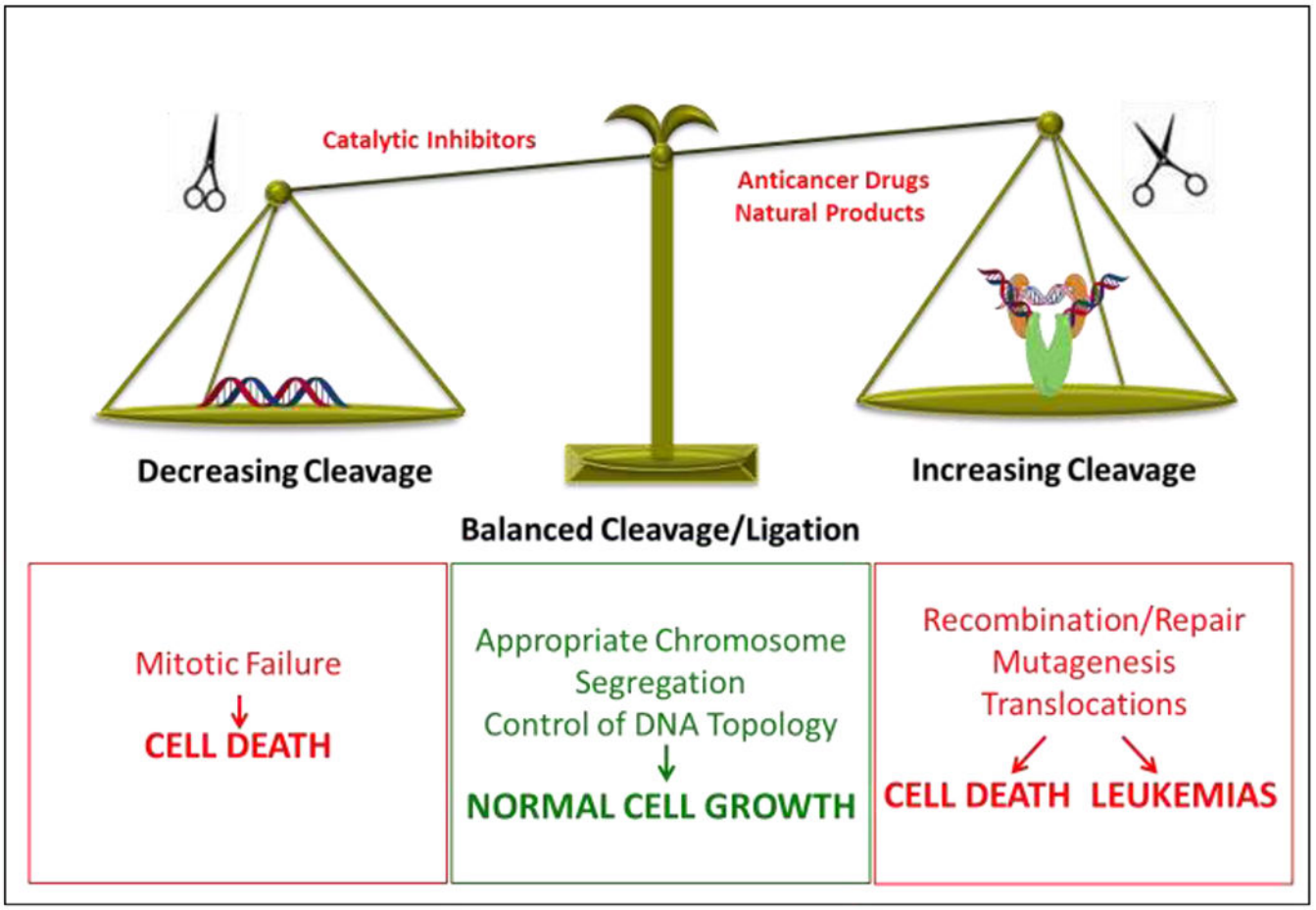


**Figure 3.**

Balancing of topoisomerase II-mediated DNA cleavage. A balanced level of topoisomerase II-DNA cleavage complexes is required for the enzyme to perform its critical cellular functions (middle). If the level of topoisomerase II-DNA cleavage complexes falls too low (left), cells are unable to untangle daughter chromosomes after DNA replication and ultimately die of mitotic failure. If the level of cleavage complexes becomes too high (right), the actions of DNA tracking systems can convert these transient complexes to permanent double-stranded breaks. The resulting DNA breaks, as well as the inhibition of essential DNA processes, initiate recombination/repair pathways that can generate chromosome translocations and other DNA aberrations. If the strand breaks overwhelm the cell, they can trigger apoptosis. This is the basis for the actions of several widely prescribed anticancer drugs and natural products that target topoisomerase II. If the concentration of topoisomerase II-mediated DNA strand breaks is too low to overwhelm the cell, mutations or chromosomal aberrations may be present in surviving populations. In some cases, exposure to topoisomerase II poisons has been associated with the formation of specific types of leukemias.

| Interfacial Poisons | Covalent Poisons |
|---|---|
| Act non-covalently at the active site and block ligation | Covalently adduct topoisomerase II at sites distal to the catalytic core |
| Enhance DNA cleavage when incubated with the enzyme prior to the addition of DNA | Inhibit topoisomerase II when incubated with the enzyme prior to the addition of DNA |
| Unaffected by reducing agents | Activity is blocked by reducing agents |
| (Examples: etoposide, doxorubicin, genistein, etc.) | (Examples: curcumin, EGCG, benzoquinone, etc.) |

**Figure 4.**

Mechanisms of topoisomerase II poisons. The two distinct mechanisms, interfacial and covalent, by which compounds can interact with the enzyme-DNA complex to enhance DNA cleavage are described.

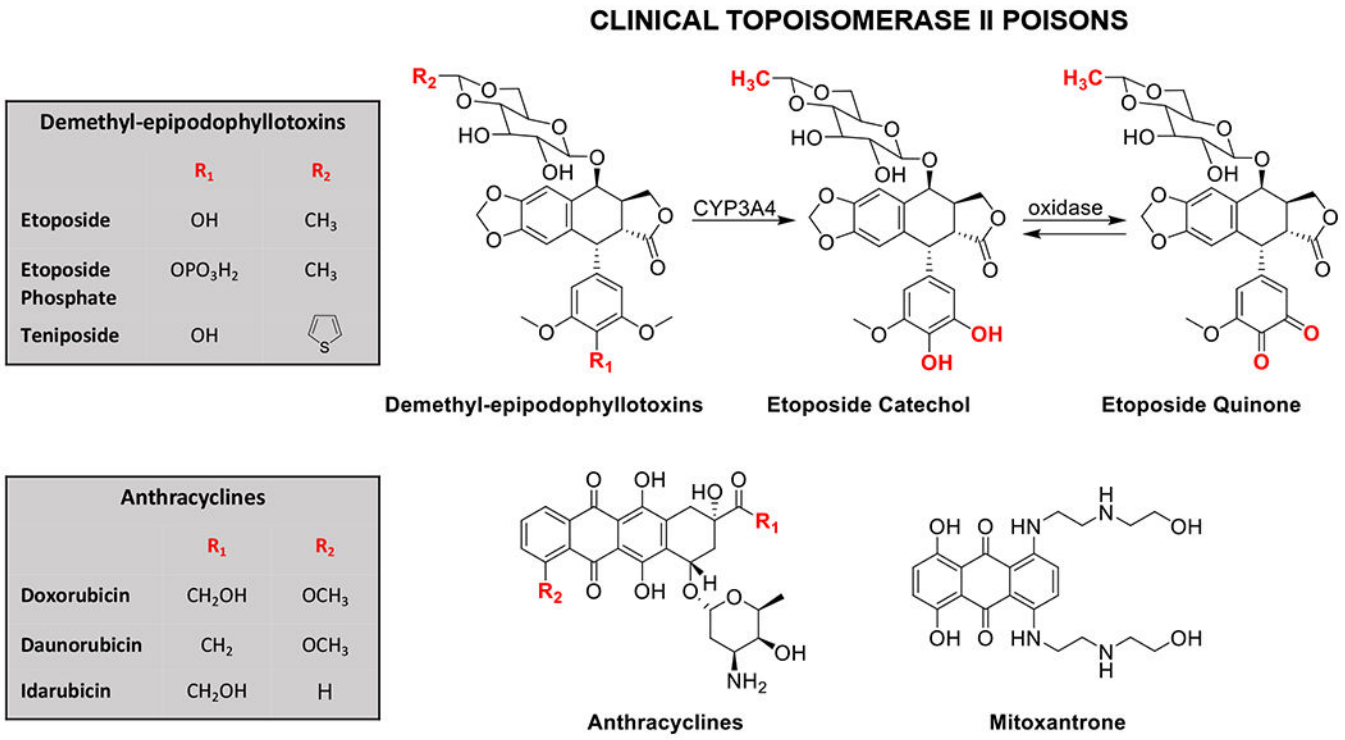


**Figure 5.**

Clinical topoisomerase II poisons. The demethyl-epipodophyllotoxins (etoposide, etoposide phosphate, and teniposide), the anthracyclines (doxorubicin, daunorubicin, and idarubicin), and the anthracenedione mitoxantrone target topoisomerase II and are approved for clinical use in the United States. A scheme for the metabolism of etoposide by cytochrome P450 and oxidase is shown (top right).

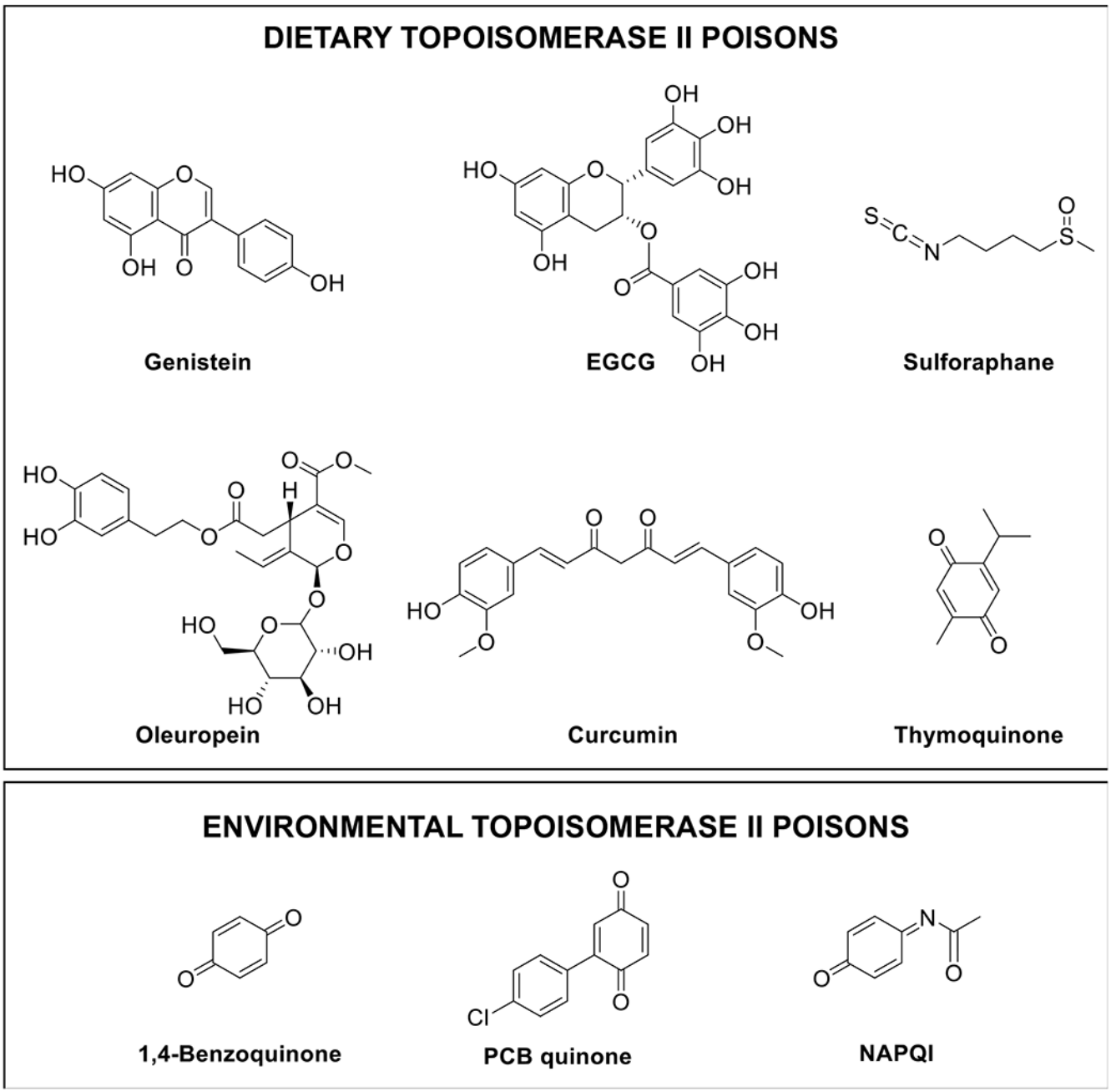


**Figure 6.**

Dietary and environmental topoisomerase II poisons. A number of dietary and environmental compounds can act as topoisomerase II poisons. Included on the list of dietary natural products are: genistein (soy), EGCG [(−)-epigallocatechin gallate; green tea], sulforaphane (cruciferous vegetables), oleuropein (olives), curcumin (turmeric), thymoquinone (black seed). On the list of environmental chemicals are: 1,4-benzoquinone (benzene metabolite), PCB (polychlorinated biphenyl) quinone (industrial fluid), and NAPQI (N-acetyl-*p*-benzoquinone imine; acetaminophen metabolite).